Modestobacter altitudinis sp. nov., a novel actinobacterium isolated from Atacama Desert soil

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Abstract

Three presumptive *Modestobacter* strains isolated from a high altitude Atacama Desert soil were the subject of a polyphasic study. The isolates, strains 1G4^T, 1G51 and 1G52, were found to have chemotaxonomic and morphological properties that were consistent with their assignment to the genus *Modestobacter*. They formed a well supported clade in *Modestobacter* 16S rRNA gene trees and were most closely related to the type strain of *'Modestobacter excelsi'* (99.8–99.9% similarity). They were also closely related to the type strains of *Modestobacter caceresii* (99.6% similarity), *Modestobacter italicus* (99.7–99.9% similarity), *Modestobacter lacusdianchii* (98.4–99.2% similarity), *Modestobacter marinus* (99.4–99.5% similarity) and *Modestobacter roseus* (99.3–99.5% similarity), but were distinguished from their closest relatives by a combination of phenotypic features. Average nucleotide identity and digital DNA:DNA hybridization similarities drawn from comparisons of draft genome sequences of isolate 1G4^T and its closest phylogenetic neighbours mentioned above, were well below the threshold used to assign closely related strains to the same species. The close relationship between isolate 1G4^T and the type strain of *M. excelsi* was showed in a phylogenomic tree containing representative strains of family *Geodermatophilaceae*. The draft genome sequence of isolate 1G4^T (size 5.18 Kb) was shown to be rich in stress related genes providing further evidence that the abundance of *Modestobacter* propagules in Atacama Desert habitats reflects their adaptation to the harsh environmental conditions prevalent in this biome. In light of all of these data it is proposed that the isolates be assigned to a novel species in the genus *Modestobacter*. The name proposed for this taxon is *Modestobacter altitudinis* sp. nov., with isolate 1G4^T (=DSM 107534^T=PCM 3003^T) as the type strain.

The actinobacterial genus *Modestobacter* [1] currently encompasses nine validly named and one effectively published species which share a broad range of chemotaxonomic, morphological and physiological features [2-4] and form a well supported 16S rRNA gene clade which distinguish them from corresponding clades of other genera classified in the family *Geodermatophilaceae* [5,6] of the order *Geodermatophilales* [7]. *Modestobacter* strains are associated with extreme biomes as exemplified by *Modestobacter multiseptatus*, the type species of the genus, isolated from soils from the Asgard Range in Antarctica [1], *Modestobacter italicus*, *Modestobacter lapidis* and *Modestobacter muralis* from deteriorated sandstone [4,8], *Modestobacter marinus* from deep-sea sediment [9] and *Modestobacter caceresii* and *'Modestobacter excelsi'* from hyper-arid Atacama Desert soils [3,10]. Culture-independent studies show that *Modestobacter* strains are key components of Atacama Desert soils [11,12] characterised by scarcity of liquid water, low concentrations of organic carbon and high solar irradiation [13-15]. In light of such considerations it is interesting that the genomes of the type strains of *M. caceresii* and *'M. excelsi'* are rich in stress genes that provide an insight into how these organisms have adapted to the prevailing environmental conditions in hyper-arid Atacama Desert soils [3,10]. Moreover, genomes of representative type strains of the family *Geodermatophilaceae* revealed many biosynthetic gene clusters (BGCs) related to the biosynthesis of secondary metabolites, as exemplified by the genomes of the type strains of *Blastococcus atacamensis* [16], *Geodermatophilus chilensis* [17], *M. caceresii* [10] and '*M. excelsi'* [3].

The present study, a continuation of our previous studies on actinobacterial diversity in Atacama Desert habitats [12,13,18], was designed to establish the taxonomic status of three presumptive *Modestobacter* strains isolated from a high altitude, hyper-arid soil of the Central-Andes in Chile. The strains, isolates 1G4^{T}, 1G51 and 1G52, were compared with reference strains of *Modestobacter* species using an combination of genomic, genotypic and

phenotypic properties. The isolates formed a new species within the genus *Modestobacter*, the proposed name for which is *Modestobacter altitudinis* sp. nov. with isolate 1G4^T as the type strain.

Isolates 1G4^T, 1G51 and 1G52 were recovered from a hyper-arid, surface soil sample (2 cm depth, pH 7.1, 360 mV, 0 % moisture content, 1.7 % organic matter content; [19]) collected at a high altitude (3018m above sea level) on Cerro Chajnantor (23° 04' 39''S/67° 57' 43'' W), east of San Pedro de Atacama in Chile [11] using a standard dilution plate procedure [3]. Aliquots (100 µl) of 10^{-1} and 10^{-2} dilutions prepared in ¼ strength Ringer's solution (Oxoid) prepared from a gram of the soil sample were spread, in triplicate, over dried plates of Gauze's No. 1 agar [20,21] supplemented with cycloheximide and nystatin (each at 50 µg ml^{-1}) prior to incubation at 28 °C for 21 days. Colonies of the three isolates were checked for purity on Gauze's agar plates and maintained on slopes of modified Bennett's agar [22] at room temperature and as cell suspensions in 20%, v/v glycerol at −80 °C. Similarly, strains of *M. caceresii* KNN 45-2b^T, *'M. excelsi'* DSM 107535^T, *M. italicus* BC 501^T, *Modestobacter lacusdianchii* KCTC 39600^T [23], *M. lapidis* MON 3.1^T, *M. marinus* DSM 45201^T, *M. muralis* MDVD1^T, *M. multiseptatus* DSM 44406^T, *Modestobacter roseus* DSM 45764^T [24] and *Modestobacter versicolor* DSM 16678^T [25] were maintained on GYM *Streptomyces* medium (DSMZ medium 65) at room temperature and as cell suspensions in 20%, v/v glycerol at −80 °C.

Biomass for most of the chemotaxonomic and molecular systematic analyses were harvested from 500 ml of modified Bennett's broth cultures (pH 7.5) which had been shaken at 150 rpm at 28 °C for 7 days following inoculation with 1 ml of each isolate prepared in modified Bennett's broth under the same conditions. Harvested cells were washed three times in sterile distilled water; those for the chemotaxonomic studies were freeze dried and the ones for the 16S rRNA gene sequencing and genomic analyses were kept at room temperature. Biomass

for the fatty acid analyses was scrapped from peptone-yeast extract-glucose-vitamins agar (PYGV, DSM medium 621) plates following incubation for 16 days at 20 °C.

The isolates were examined for chemotaxonomic, cultural and morphological properties known to be of value in the classification of *Modestobacter* species [3,4]. Motility and micromorphological features were acquired according to Trujillo *et al.* [8] and cultural properties recorded from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agar (International *Streptomyces* Project [ISP] media 1–7 respectively; [26]) plates following incubation at 28 ºC for 14 days; colony colours were determined by comparison against colour charts [27]. In turn, chemotaxonomic properties of the isolates were established using standard procedures. Whole-cell amino acids and sugars prepared after Lechevalier and Lechevalier [28] were analysed by thin-layer chromatography (TLC), as described by Staneck and Roberts [29]; menaquinones extracted from freeze dried cells with methanol [30] were separated by high-performance liquid chromatography (HPLC) [31] while polar lipids were extracted from freeze dried cells, separated by two-dimensional TLC and identified after Minnikin *et al.* [32] using modifications introduced by Kroppenstedt and Goodfellow [33]. Fatty acids extracted from the isolates and the type strains of *M. caceresii, 'M. excelsi', M. italicus, M. marinus* and *M. roseus* harvested under the same growth conditions were analysed using the Sherlock Microbial Identification (MIDI) system version 5 [34] and the resultant acids identified using the ACTIN6 database, as described previously [3].

The chemotaxonomic, cultural and morphological properties of the isolates were consistent with their classification in the genus *Modestobacter* [1-4,8]. The isolates were aerobic, Gram-stain-positive, non-motile, formed short rods and coccoid-shaped cells which tended to remain aggregated (**Fig. S1**), produced whole-organism hydrolysates rich in *meso*-A_2pm, glucose and ribose (diagnostic sugars), MK-9(H_4) as the predominant menaquinone (81-93.1%) and polar

lipid patterns containing diphosphatidylglycerol, glycophosphatidylinositol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol and phosphatidylinositol, as exemplified in **Figure S2**.

The isolates grew particularly well on ISP media 2 and 6 producing black colonies on the former and dark brown or light-olive ones the latter (**Table 1**).

It is worth mentioning that in previously studies [8,10,24] glycophosphatidylinositol (GPI) was annotated as phosphatidylinositol mannoside (PIM); this compound has been found in representatives of *Modestobacter* species, apart from *M. multiseptatus* [1,4]. Consequently, the polar lipid profiles of isolates 1G4^T, 1G51 and 1G52 accord with those found in the type strains of most *Modestobacter* species [3-4,10]. Similarly, the predominance of MK-9(H_4) in the isolates agrees with data previously recorded for members of the genus *Modestobacter* [1-4]. The cellular fatty acid profiles of the isolates contained variable proportions of $C_{17:1}$ ω8c (4.5-17.3% of total fatty acids), $C_{18:1}$ ω9c (15.6-26.2%), iso-$C_{15:0}$ (5.0-16.5%) and iso-$C_{16:0}$ (11.2-41.9%), quantitative differences were also recorded for components found in lesser proportions (**Table 2**). $C_{17:1}$ ω8c has also been detected in '*M. excelsi*' DSM 107535^T and *M. marinus* DSM 45201^T and $C_{18:1}$ ω9c in the type strains of *M. caceresii*, '*M. excelsi'*, *M. marinus* and *M. roseus* albeit in lesser proportions than in the isolates though it was the predominant component in *M. italicus* BC 501^T. Similarly, smaller proportions of $C_{16:0}$ were found in all of the reference strains with only a trace amount found in *M. roseus* DSM 45764^T.

Genomic DNA was extracted from each of the isolates using a GenElute^{TM} Bacterial Genomic Kit (Sigma-Aldrich) following the instructions of the manufacturer and PCR-mediated amplifications of the 16S rRNA genes carried out, as described by Golinska *et al.* [35,36]. PCR-products were purified using a PCR purification kit (Qiagen) following the protocol of the manufacturer, checked for quality with a NanoDrop 2000 spectrophotometer (Thermo

Fisher Scientific) and sequenced at the Institute of Biochemistry and Biophysics (IBB) of the Polish Academy of Sciences, Warsaw, Poland using an ABI3730xl Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequences of isolates 1G4^T, 1G51 and 1G52 (1526, 1398 and 1394 nucleotides [nt], respectively) were deposited in the GenBank sequence database (accession numbers MH430521, MH430522 and MN065570, respectively). The sequences were compared with corresponding sequences of *Modestobacter* type strains using the Eztaxon server [37]. The 16S rRNA gene sequence similarities were calculated using PHYDIT version 1.0 software [38].

Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic trees based on the 16S rRNA gene sequences were inferred using the genome-to-genome distance calculator (GGDC) web server [39] adapted to single genes and multiple sequence alignments generated using ClustalW [40]. The ML tree was inferred from the alignments with RAxML [41] using rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion [42]. The MP tree was inferred from the alignments with the Tree Analysis using the New Technology (TNT) program [43] with 1000 bootstraps together with tree bisection and reconnection branch swapping and 10 random sequence replicates. The sequences were checked for computational bias using the x^2 test implemented in PAUP* [44]. A third tree was generated with the neighbour-joining [45] algorithm from the MEGA 7 software package [46]; an evolutionary distance matrix for the neighbour-joining analysis was prepared using the two-parameter model of Kimura [47]. The topologies of the inferred evolutionary trees were evaluated by bootstrap analyses [48] based on 1,000 replicates. The root positions of unrooted trees were estimated using the sequence of *Cumulibacter manganitolerans* 2-36^T (Genbank accession number: KX602199). BOX-PCR fingerprints of the isolates were prepared from extracted DNA using the BOXAIR primer [49] and the experimental conditions described by Trujillo *et al.* [50].

The isolates formed a well-supported clade in the *Modestobacter* 16S rRNA gene tree (**Fig. 1, Fig. S3**). Strains 1G4^T and 1G52 have identical 16S rRNA gene sequences and share a 99.9% similarity with isolate 1G51 which corresponds to 2 and 1 nt differences at 1411 and 1387 locations, respectively. The isolates were most closely related to *'M. excelsi'* DSM 107535^T sharing 16S rRNA gene sequence similarities with the latter of 99.9% (1G4^T), 99.8% (1G51) and 99.9% (1G52), values equivalent to 1 and 3 nt differences. They were also closely related to *M. italicus* BC 501^T (99.7 - 99.9% similarity), *M. caceresii* KNN 45-2b^T (99.6% similarity), *M. marinus* DSM 45201^T (99.4 – 99.5% similarity), *M. roseus* DSM 45764^T (99.3 - 99.5 % similarity) and *M. lacusdianchii* KCTC 39600^T (98.4-99.2 % similarity). All of these similarity values are above the 98.7% threshold used to recognise novel prokaryotic species [51]. In contrast, the isolates were separated from the remaining *Modestobacter* type strains sharing 16S rRNA gene similarities with them within the range 97.8 to 98.7 %, values corresponding to between 18 and 31 nt differences. The isolates showed diverse BOX-PCR profiles indicating that they are not clones (**Fig. 2**).

Comparative analyses of whole genome sequences are proving to be effective in establishing relationships between closely related species that are difficult to resolve using conventional taxonomic methods [52-54]. In this study, a single colony of isolate 1G4^T was used to inoculate 50 ml of modified Bennett's broth [22] prior to incubating at 28 ºC for 72 hours in a shaking incubator at 150 rpm. Genomic DNA extracted from 1.5 ml of the resultant culture was sequenced at MicrobesNG (http://www.microbesng.uk) using a MiSeq instrument (Illumina). The reads were assembled into contigs with SPAdes 3.6.2 software [55] and contigs under 300 bp discarded. The draft genome assembly was annotated using the RAST server [56,57] with default options and submitted to GenBank (accession no. SJEW00000000). The GGDC server [39,58] was used to estimate dDDH values between the draft genome sequence of strain 1G4^T and the type strains of *M. caceresii* (GenBank

accession number: JPMX00000000), *'M. excelsi'* (GenBank accession number: SJEX00000000), *M. italicus* (GenBank accession number: FO203431), *M. lacusdianchii* (unpublished, provided by Montero-Calasanz), *M. marinus* (IMG genome ID: 2820994125) and *M. roseus* (IMG genome ID: 2585427561), phylogenetic relatives that showed similarities higher than 98.7 % based on 16S rRNA gene sequences. In turn, the OrthoANIu algorithm from the ANI Calculator [59,60] was used to calculate ANI values between isolate 1G4^T and the type strains of its closest relatives, with the exception of the ANI value between isolate 1G4^T and *M. marinus* DSM 45201^T which was provided by Vartul Sangal from Northumbria University, UK.

The draft genome sequence of isolate 1G4^T contained 161 contigs giving a total size of 5,175,439 bp with a DNA G+C content of 73.7 mol%. The genome contained 52 RNA genes and was annotated to include 5182 protein coding sequences which were assigned to 324 subsystems. Most of the categories with coding sequences classified into subsystems were amino acids and derivatives (15.7%), followed by carbohydrates (15.3%), proteins (10.1%), cofactors, vitamins, prosthetic groups and pigments (9.9%) as well as fatty acids, lipids and isoprenoids (8.4%) (**Fig. 3**).

The dDDH values between isolate 1G4^T and the type strains of *M. caceresii, 'M. excelsi'*, *M. italicus*, *M. lacusdianchii*, *M. marinus* and *M. roseus* were 35.6, 47.6, 41.4, 27.2, 35.8 and 32.5%, respectively, values well below the recommended cut-off point of ≥ 70% used to assign strains to the same species [61]. Similarly, ANI values of 83.4, 92.2, 90.5, 83.8, 84.1 and 82.2 % observed between the genomes of isolate 1G4^T and the reference strains cited above are well below the 95-96% threshold used to distinguish between members of closely related species [62,63].

The genome sequences of isolate 1G4^T and the type strains of *M. lacusdianchii* and *M. multiseptatus* were uploaded on to Type (Strain) Genome Server (TYGS) [58] and compared

against all of the type strain *Geodermatophilaceae* genomes available in the TYGS database using the MASH algorithm which allows a fast approximation of intergenomic relatedness between strains [64]. A phylogenomic tree was inferred with FastME 2.1.4 [65] from GBDP distances calculated from the genome sequences, branch lengths were scaled using GBDP distance formula d_5 [39], numbers above branches are GBDP pseudo-bootstrap support values based on 100 replications. It can be seen from the phylogenomic tree that the isolate forms not only a distinct branch in the *Modestobacter* clade but is most closely related to the '*M. excelsi*' and lesser so to the type strains of *M. italicus* and *M. versicolor* (**Fig. 4**).

The isolates and *'M. excelsi'* DSM 107535^T, their nearest phylogenetic neighbour, were examined for a broad range of phenotypic properties. They were tested for their ability to grow over a range of pH values (pH 4.0–12.0 at 0.5 unit intervals using phosphate buffers) and temperatures (4, 10, and then at 5 ºC intervals up to 45 ºC) and in the presence of various concentrations of sodium chloride (0–10% NaCl, w/v) using ISP 2 agar as the basal medium [26]. Apart from the temperature tests, these features were recorded at 28 ºC after incubation for 2 weeks. The biochemical and degradation tests were done as recommended by Williams *et al*. [66]. All of these tests were carried out in duplicate using a standard inoculum equivalent to 5.0 on the McFarland scale [67]. Enzyme profiles were established using API-ZYM kits (bioMerieux) following the instructions of the manufacturer. The isolates, and all of the *Modestobacter* type strains, including *'M. excelsi'* DSM 107535^T, were examined for their capacity to oxidise a broad range of carbon and nitrogen compounds and to show resistance to inhibitory compounds using GEN III microplates in an Omnilog device (BIOLOG Inc., Haywood, USA) following incubation of duplicated cultures at 28 ºC for 7 days. The strains were grown on GYM *Streptomyces* agar plates at 28 ºC and cell suspensions prepared in a viscous inoculating fluid (IF C) at around 83% transmittance (T); the microplates were

inoculated by following the protocol of the manufacturer. The resultant data were exported and analysed using the opm package for R v.1.0.6 [68,69].

The duplicated cultures of the isolates gave the same results for the API-ZYM, biochemical, degradation and physiological tests though variable results were recorded for some of the Biolog tests, as shown in **Table 1.** It is apparent that the isolates have many phenotypic properties in common though some features can be weighted to distinguish between them providing further evidence that they are not clones. Isolate 1G52, for instance, metabolised many more carbon and nitrogen compounds than isolates 1G4^{T} and 1G51; the former unlike the latter produced α-fucosidase, α-galactosidase, lipase (C14) and α-mannosidase and was unable to oxidise dextrin, β-*methyl*-D-glucoside, butyric acid, α-*keto*-glutaric acid or L-malic acid and grow in the presence of D-serine.

With few exceptions the duplicated cultures gave the same results in the Biolog tests, as shown in **Table 3**. It is also evident from this Table that the isolates can be distinguished from the type strains of all of the *Modestobacter* species, including those of its closest phylogenetic neighbours, based on a wide range of phenotypic properties. The isolates, unlike *'M. excelsi'* DSM 107535^{T}, its closest phylogenetic neighbour, oxidised *p-hydroxy*-phenylacetic acid, quinic acid and D-salicin, degraded pectin and gelatin and grew in the presence of aztreonam. In contrast, the *'M. excelsi'* strain, unlike the isolates, oxidised L-fucose, α- and β-*hydroxy*-butyric acids, α-*keto*-butyric acid, citric acid, D-galacturonic acid and D-glucuronic acid. In addition, the isolates can be distinguished from the *'M. excelsi'* strain based on their ability to reduce nitrate. In contrast, *'M. excelsi'* DSM 107535^{T}, unlike the isolates, was positive for α-chymotrypsin and trypsin. The isolates, unlike *M. italicus* BC 501^{T}, its closest neighbour with a validly published name, oxidised D-fructose-6-phosphate, glucuronamide, D-raffinose, acetic acid, acetoacetic acid, quinic acid and *p-hydroxy*-phenylacetic acid, degraded gelatin and pectin and grew at pH 6. In contrast, the *M. italicus* strain, unlike the isolates oxidised

glycerol, D-mannitol and D-gluconic acid. Similar combinations of phenotypic features can be used to separate the isolates from the remaining *Modestobacter* type strains.

The genome of isolate 1G4^T was examined for natural product-biosynthetic gene clusters (NP-BGCs) using anti-SMASH software [70] and gene sequences encoding proteins involved in stress responses were detected using the SEED server [71]. The draft genome of the isolate contained NP-BGCs coding for type 2 and type 3 polyketide synthases, betalactone and terpenes thereby underpinning corresponding results on the type strains of *M. caceresii* [10] and *'M. excelsi'* [3]. In turn, many of the stress related genes were shown to be linked with carbon starvation, oxidative and osmotic stress, resistance to UV radiation and temperature fluxes, as exemplified by genes encoding for carbon starvation protein A (CstA), which promotes peptide uptake [72-74]; *sox* genes associated with responses to oxidative stress [75,76]; *KatE* and *uvrD* genes linked to protection against reactive oxygen and UV-radiation [77]; *dnaK* and *dnaJ*, *hrcA*, *grpE* and *Hsp* genes related to responses to heat shock [78]; a family of proteins associated with cold shock responses [79] and *coxE, coxD* and *coxG* genes along with a *coxSML* cluster coding for utilisation of carbon monoxide indicating that the isolate may have a chemolithoautotrophic lifestyle [80]. Similar patterns of stress-related genes have been detected in the genomes of the type strains of the *B. atacamensis* [16], *G. chilensis* [17], *M. caceresii* [10] and *'M. excelsi'* [3]. These results go someway towards accounting for the high numbers of *Geodermatophilaceae* propagules present in Atacama Desert habitats where extreme environmental conditions prevail [11,19]. Indeed, these data lend support to the view that the competitive success of these organisms in extreme, sparsely populated biomes may be a function of stress resistance not antibiosis [12].

It can be concluded from this wealth of genotypic and phenotypic data that isolates 1G4^T, 1G51 and 1G52 belong to a novel species of *Modestobacter* and that isolate 1G4^T can be distinguished from all of its closest phylogenetic neighbours by low ANI and dDDH values. It

is, therefore, proposed that this novel taxon be recognised as *Modestobacter altitudinis* sp. nov.

Description of *Modestobacter altitudinis* sp. nov.

Modestobacter altitudinis (al.ti.tu□di.nis. L. gen. n. *altitudinis* of a high place).

Aerobic, Gram-stain-positive, non-motile actinobacteria that form short rods and coccoid-like elements. Grows from 10 to 35 °C (type strain), optimally around 28 °C, from pH range 5.0-9.0 (type strain), optimally 7.5-8.0, and in presence of up to 8% NaCl, w/v (type strain). Aesculin is hydrolysed but not allantoin or urea. Nitrate is reduced. Tweens 40 and 60 are degraded, but not adenine, casein, chitin, elastin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine, uric acid, xanthine or xylan. Positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α- and β-glucosidases, leucine arylamidase and valine arylamidase, but negative for α-chymotrypsin, β-glucuronidase and trypsin. Oxidises D-fructose-6-phosphate, D-glucose, glucuronamide, D-raffinose, D-salicin, actetic acid, acetoacetic acid, *p-hydroxy*-phenylacetic acid and quinic acid, but not D-arabitol, D- or L-fucose, N-*acetyl*-D-galactosamine, D-galactose, N-*acetyl*-D-glucosamine, glycerol, *myo*-inositol, α-D-lactose D-mannitol, N-*acetyl*-β-mannosamine, α- and β-*hydroxy*-butyric acids, α-*keto*-butyric acid, γ-amino-*n*-butyric acid, citric acid, L-galactonic acid-γ-lactone, D-galactouronic acid, D-gluconic acid, D-glucuronic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, D-saccharic acid and L-alanine, D-aspartic acid, L-histidine, L-pyroglutamic acid or D-serine and inosine. Resistant to aztreonam, nalidixic acid, rifamycin SV, lithium chloride, potassium tellurite and sodium lactate (1%). Whole cell hydrolysates contain *meso*-diaminopimelic acid, glucose and ribose, the major fatty acids are $C_{17:1}$ ω8c, $C_{18:1}$ ω9c, iso- $C_{16:0}$, MK-9(H_4) is the predominant menaquinone, and phosphatidylethanolamine is the diagnostic phospholipid. The

genome size of the type strain is around 5.2 Mbp and its genomic DNA G+C content 73.7 mol%.

The type strain 1G4^T (DSM 107534^T= PCM 3003^T) was isolated from a surface soil sample collected from Cerro Chajnantor, near San Pedro de Atacama, Chile. The accession number of 16S rRNA gene sequence of isolate 1G4^T is MH430521 and the accession number of its genome sequence is SJEW00000000.

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Conflicts of interest

The authors have no conflicts of interest.

Ethical statement

The authors have not carried out any studies involving human participants or animals.

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Legend for Figures:

Fig. 1. Maximum-likelihod and maximum-parsimony tree based on nearly complete 16S rRNA gene sequences (1390-1541 nucleotides) showing relationships between isolates 1G4^T, 1G51 and 1G52 and between them and the type strains of *Modestobacter* species. The numbers above the branches are bootstrap support values when larger than 70% from ML (left) and MP (right). Bar; 0.007 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^T as the outgroup.

Fig. 2. BOX PCR fingerprint patterns of genomic DNA extracted from the isolates.

Fig. 3. The subsystem category distribution statistics for isolate1G4^T based on the SEED database. Bar chart shows the percentage of subsystem coverage (green bar corresponds to the percentage of proteins involved). The pie chart with legend shows the fraction and count (parenthesis in legend) of each subsystem feature.

Fig 4. Phylogenomic tree showing relationship between isolate 1G4^T and type strains of species classified in the family *Geodermatophilaceae*. The numbers above the branches are GBDP pseudo-bootstrap support values > 70 % from 100 replications with an average branch support of 94.7 %. The tree was rooted at the midpoint [82].

Table 1. Cultural and phenotypic properties that distinguish between the isolates.

	Isolates					
Characteristics	**1G4^T**		**1G51**		**1G52**	
Growth		Colony colour		Colony colour		Colony colour
Glycerol-asparagine agar (ISP 5)	+	Dark olive	+	Light yellow	+	Dark olive
Inorganic salts starch agar (ISP 4)	+	Black	+	Moderate olive brown	+	Black
Oatmeal agar (ISP 3)	++	Black	++	Black	++	Black
Peptone – yeast extract iron agar (ISP 6)	+++	Dark brown	+++	Light olive	++	Light olive
Tryptone-yeast extract agar (ISP 1)	++	Black	++	Black	+++	Black
Tyrosine agar (ISP 7)	+	Black	+	Pale orange yellow	+	Light olive
Yeast extract - malt extract agar (ISP 2)	+++	Black	+++	Black	++	Black
Hydrolysis of:						
Arbutin		+		-		+
Degradation of:						
Tween 20		-		+		-
API-ZYM tests:						
Cystine arylamidase		+		-		-
α-Fucosidase		-		-		+
α-Galactosidase		-		-		+
β-Galactosidase		+		-		+
N-*acetyl*-β-Glucosaminidase		+		+		-
Lipase (C14)		-		-		+
α-Mannosidase		-		-		+
Naphthol-AS-BI-phosphohydrolase		+		+		-
BIOLOG GEN III microplate tests:						
Oxidise:						
D-Cellobiose		-		-		+
Dextrin		+		+		-
D-Fructose		-		+		+
β-Gentiobiose		-		-		+
3-*O-methyl*-D-Glucose		-		+		+
D-Glucose-6-phosphate		-		+		+
β-*methyl*-D-Glucoside		-		+		-
D-Maltose		-		-		+
D-Mannose		-		-		+
D-Melibiose		-		-		+
L-Rhamnose		-		-		+
D-Sorbitol		+		-		+
D- Stachyose		-		-		+
D-Sucrose		-		-		+
D-Trehalose		-		+		+
D-Turanose		-		-		+
L-Arginine		-		-		+
L-Aspartic acid		-		-		+
Glycyl-L-proline		-		-		+
L-Glutamic acid		-		-		+

L-Serine	+	-	+
Bromo-succinic acid	-	+	-
Butyric acid	+	+	-
α-*keto*-Glutaric acid	+	+	-
L-Lactic acid	+	-	+
L-Malic acid	+	+	-
Methyl-pyruvate	+	-	+
N-*acetyl*-Neuraminic acid	-	-	+
Propionic acid	+	+	-
Growth in presence of inhibitory compounds:			
Fusidic acid	+	+	-
Guanidine hydrochloride	+	+	-
Lincomycin	+	+	-
Minocycline	+	+	-
Niaproof	+	+	-
Troleandomycin	+	+	-
Vancomycin	+	+	-
D-serine	+	+	-
Sodium bromate	+	+	-
Sodium formate	+	+	-
Tetrazolium violet	+	+	-
Tetrazolium blue	+	+	-
Temperature growth range (°C)	10-35	10-35	10-30
pH growth range	5.0-9.0	5.0-9.0	6.0-8.0
NaCl tolerance (% w/v)	0-8	0-8	0-3

Key: Growth: +++, abundant; ++, good; +, weak.

Physiological tests: +, positive; -, negative

All of the isolates reduced nitrate, hydrolysed aesculin but not allantoin or urea; degraded Tweens 40 and 60 but, not adenine, casein, chitin, elastin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine, uric acid, xanthine or xylan. They were positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α- and β-glucosidases, leucine arylamidase, valine arylamidase and negative for trypsin, α-chymotrypsin and β-glucuronidase (API-ZYM tests) and oxidised D-fructose-6-phosphate, D-glucose, glucuronamide, D-raffinose and D-salicin (sugars), acetic acid, acetoacetic acid, *p-hydroxy*-phenylacetic acid and quinic acid (organic acids), degraded gelatin, pectin and Tween 40 (polymers), and grew in the presence of aztreonam, lithium chloride, nalidixic acid, potassium tellurite, rifamicin SV, sodium chloride (1% w/v), sodium lactate (1% w/v) and at pH 6. In contrast, they did not oxidise L-alanine, D-aspartic acid, L-histidine, L-pyroglutamic acid or D-serine (amino acids), D-arabitol, D- or L-fucose, N-*acetyl*-D-galactosamine, D-galactose, N-*acetyl*-D-glucosamine, glycerol, *myo*-inositol, α-D-lactose, D-mannitol and N-*acetyl*-β-mannosamine (sugars), α- and β-*hydroxy*-butyric acid, α-*keto*-butyric acid, γ-amino-*n*-butyric acid, citric acid, L-galactonic acid-γ-lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, D-saccharic acid (organic acids) or inosine (nucleoside).

Table 2. Fatty acid profiles (%) of the isolates grown on peptone-yeast extract-glucose-vitamins agar plates after incubation for 16 days at 20 ºC. Values are percentages of total fatty acids. All data are from this study.

				***Modestobacter* strains**				
Fatty acids	**Isolate 1G4^T**	**Isolate 1G51**	**Isolate 1G52**	***M. caceresii* KNN 45-2b^T**	***'M. excelsi'* DSM 107535^T**	***M. italicus* BC 501^T**	***M. marinus* DSM 45201^T**	***M. roseus* DSM 45764^T**
$C_{14:0}$	1.6	0.8	1.6	tr	tr	tr	tr	tr
$C_{15:0}$	-	-	-	tr	-	tr	tr	2.4
$C_{16:0}$	8.4	3.5	2.9	3.1	1.6	1.0	4.1	tr
$C_{17:0}$	4.4	tr	tr	tr	tr	tr	1.1	1.7
$C_{18:0}$	3.5	1.2	2.3	-	tr	tr	tr	-
$C_{16:1}$ cis 9	-	-	-	23.5	-	5.7	-	5.4
$C_{17:0}$ cyclo	-	-	-	2.6	-	-	1.7	1.5
$C_{17:1}$ ω8c	17.3	4.5	4.7	-	3.3	-	7.6	-
$C_{17:1}$ cis 9	-	-	-	7.6	-	4.5	-	10.9
$C_{18:1}$ ω6c	2.5	tr	tr	-	-	-	-	-
$C_{18:1}$ ω9c	26.2	16.6	15.6	3.8	7.3	17.3	4.1	tr
$C_{16:0}$ 9-methyl	-	-	-	1.8	-	1.5	-	4.4
$C_{17:0}$ 10-methyl	-	-	-	1.8	-	4.4	6.0	tr
anteiso- $C_{15:0}$	1.2	2.9	5.3	tr	5.8	1.2	tr	tr
anteiso- $C_{17:0}$	1.7	3.3	8.3	tr	-	1.9	tr	tr
anteiso- $C_{17:1}$	-	-	-	-	6.3	-	-	-
anteiso- $C_{17:1}$ C	-	-	-	tr	-	tr	-	1.0
anteiso-$C_{17:1}$ ω9c	-	1.1	2.5	-	3.8	-	tr	-
iso-$C_{10:0}$	-	-	-	-	-	1.3	-	-
iso-$C_{14:0}$	tr	2.1	tr	1.7	1.4	1.4	2.3	3.1
iso-$C_{15:0}$	5.0	6.1	16.5	3.2	18.7	6.9	2.9	16.0
iso-$C_{15:1}$ G	1.6	2.8	3.6	3.1	8.1	4.4	3.0	-
iso-$C_{16:0}$	11.9	41.9	11.2	28.1	16.3	32.8	32.5	25.7
iso-$C_{16:0}$ 2OH	-	-	-	-	-	2.0	-	1.7
iso-$C_{16:1}$ G	tr	-	-	-	-	5.6	-	-
iso-$C_{16:1}$ H	-	-	1.7	14.9	5.8	-	16.7	9.4
iso-$C_{17:0}$	1.1	1.8	5.6	tr	1.7	1.9	1.4	2.4
iso-$C_{17:1}$ ω9c	-	-	-	-	5.9	-	1.7	-
Sum in feature 3	4.1	5.0	5.9	-	9.1	-	8.5	-
Sum in feature 4	-	-	-	-	-	-	-	2.6
Sum in feature 7*/**	5.5*	2.9*	3.2*	tr**	1.0*	tr**	tr*	tr**
Sum in feature 9*/**	tr**	1.1**	4.9**	tr*	-	tr*	-	-

Key: -, not detected. tr, trace (amount below 1%).

*As indicated by Montero-Calasanz *et al.* [79] summed features are groups of two or three fatty acids that are taken together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately.

Summed feature 3 comprises $C_{16:1}$ ω7c and $C_{16:1}$ ω6c; summed feature 4 iso-$C_{15:0}$ 2OH and $C_{16:1}$ t9; summed feature 7* $C_{19:0}$ cyclo ω10c and/or 19ω6; summed feature 7** $C_{18:1}$ trans 9 644 and $C_{18:1}$ trans 6 and $C_{18:1}$ cis; summed feature 9 $C_{19:0}$ cyclo C9-10 and undefined fatty acid.

Table 3. Phenotypic properties that distinguish the isolates from the type strains of *Modestobacter* species.
Strains: 1, Isolates 1G4T, 1G51 and 1G52; 2, *M. caceresii* KNN 45-2b^T; 3, *'M. excelsi'* DSM 107535^T; 4, *M. italicus* BC 501^T; 5, *M. lacusdianchii* KCTC 39600^T; 6, *M. lapidis* MON 3.1^T; 7, *M. marinus* DSM 45201^T; 8, *M. multiseptatus* DSM 44406^T; 9, *M. muralis* MDVD1^T; 10, *M. roseus* DSM 45764^T; 11, *M. versicolor* DSM 16678^T.

	***Modestobacter* strains**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
BIOLOG GEN III microplate tests Assimilation of:											
D-Fructose-6-phosphate	+	+	+	-	-	-	+	-	+	-	+
D-Fucose	-	+	-	-	-	+	-	+	-	-	+
L-Fucose	-	+	+	-	+	+	+	+	+	-	+
D-Galactose	-	+	-	-	+	+	+	+	+	-	+
N-*acetyl*-D-Galactosamine	-	-	-	-	-	+	+	+	+	+	-
N-*acetyl*-D-Glucosamine	-	+	-	-	+	+	+	+	+	+	+
Glucuronamide	+	+	+	-	-	+	-	+	-	-	-
Glycerol	-	+	-	+	+	-	+	+	+	+	+
Inosine	-	-	-	-	-	-	+	-	-	-	-
α-D-lactose	-	-	-	-	+	+	+	+	+	+	-
D-Mannitol	-	+	-	+	+	+	+	+	+	+	+
N-*acetyl*-β-D-Mannosamine	-	+	-	-	+	+	+	+	+	+	+
Myo-inositol	-	-	-	-	-	+	-	+	-	-	-
Pectin	+	+	-	-	+	-	+	-	+	+	+
D-Raffinose	+	-	+	-	+	+	-	-	+	-	-
D-Salicin	+	+	-	+	+	+	+	+	+	+	-
L-Pyroglutamic acid	-	-	-	-	-	-	+	+	-	-	-
Acetic acid	+	+	+	-	+	+	-	+	+	+	+
Acetoacetic acid	+	+	+	-	+	+	-	+	+	+	+
γ-amino-*n*-Butyric acid	-	+	-	-	-	-	-	+	-	-	+
α-*hydroxy*-Butyric acid	-	+	+	-	-	+	-	-	-	-	-
β-*hydroxy*-Butyric acid	-	+	+	-	-	-	-	+	-	-	+
α-*keto*-Butyric acid	-	+	+	-	-	+	-	-	-	+	-
Citric acid	-	-	+	-	+	-	-	+	-	-	+
D-Galacturonic acid	-	-	+	-	+	+	-	+	-	-	-
D-Gluconic acid	-	+	-	+	+	+	+	+	+	+	+
D-Glucuronic acid	-	+	+	-	-	+	-	+	-	-	+
D-Lactic acid methyl ester	-	+	-	-	-	-	-	-	-	-	+
D-Malic acid	-	-	-	-	+	-	-	+	+	+	-
Quinic acid	+	-	-	-	-	-	-	-	-	+	-
p-hydroxy-Phenylacetic acid	+	+	-	-	-	-	-	-	-	+	-
D-Saccharic acid	-	-	-	-	-	-	+	-	-	+	-
Gelatin	+	+	-	-	-	-	+	-	-	-	-
Tween 40	+	+	+	+	+	+	-	+	+	+	-
Growth in presence of inhibitory compounds:											
Aztreonam	+	+	-	+	+	+	+	-	+	+	+
Nalidixic acid	+	-	+	+	+	+	+	-	+	+	+

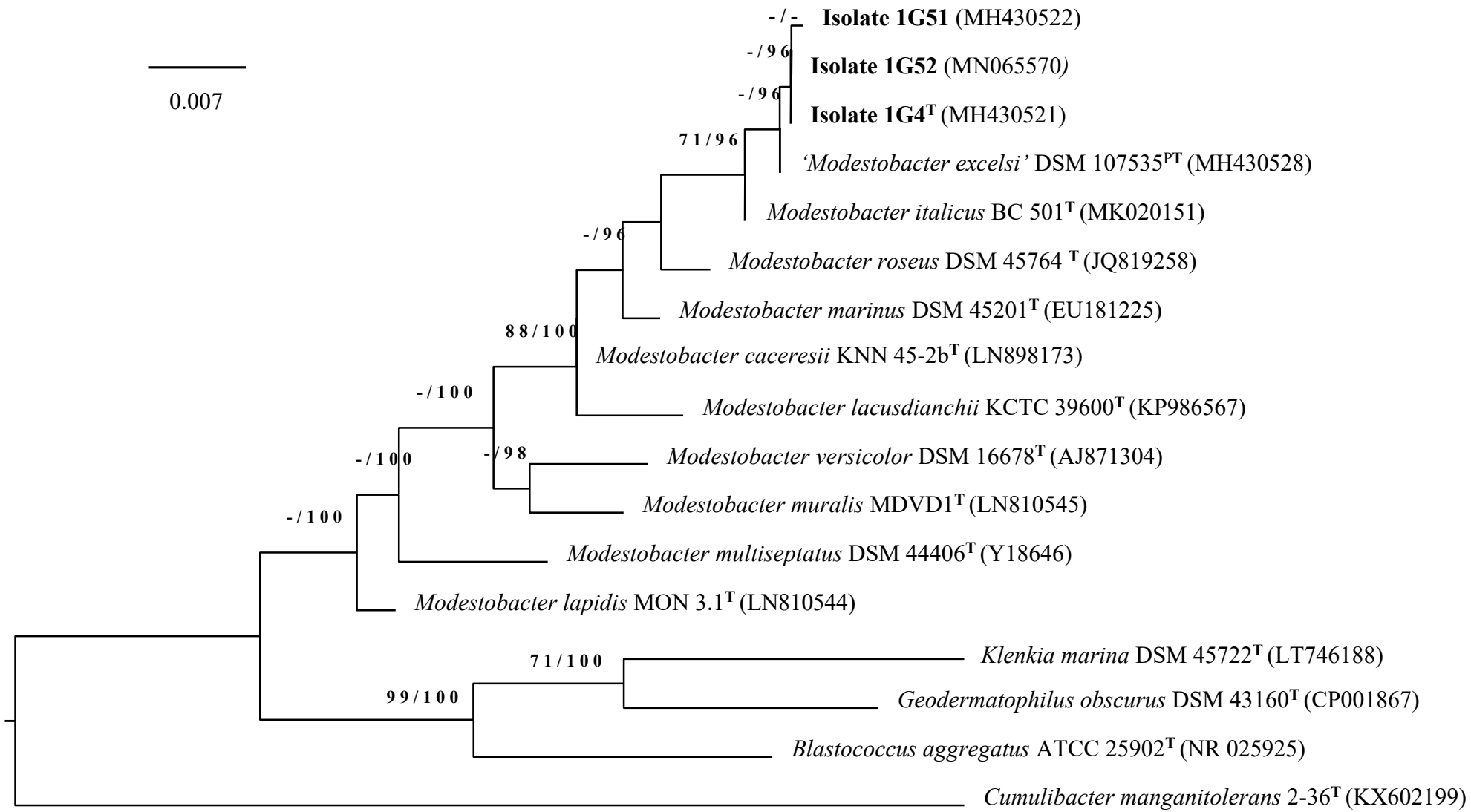
Potassium tellurite	+	-	+	+	+	-	-	-	+	+	+
Rifamycin SV	+	-	+	+	+	-	-	-	-	-	+
Sodium lactate (1% w/v)	+	-	+	+	-	-	+	-	+	+	+
growth at pH 6	+	-	+	-	-	-	+	-	-	-	-
Polar lipids*	DPG, PG, PE, PI, GPI 4L	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, GPI, 2L	DPG, PE, PI, GPI	DPG, PE, PI, PIM, PL	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, APL, GL, PL	DPG, PE, PI, GL, 2APL, 3L	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, GPI, AL, 2APL	DPG, PG, PE, PI, PL, GPI, 4L

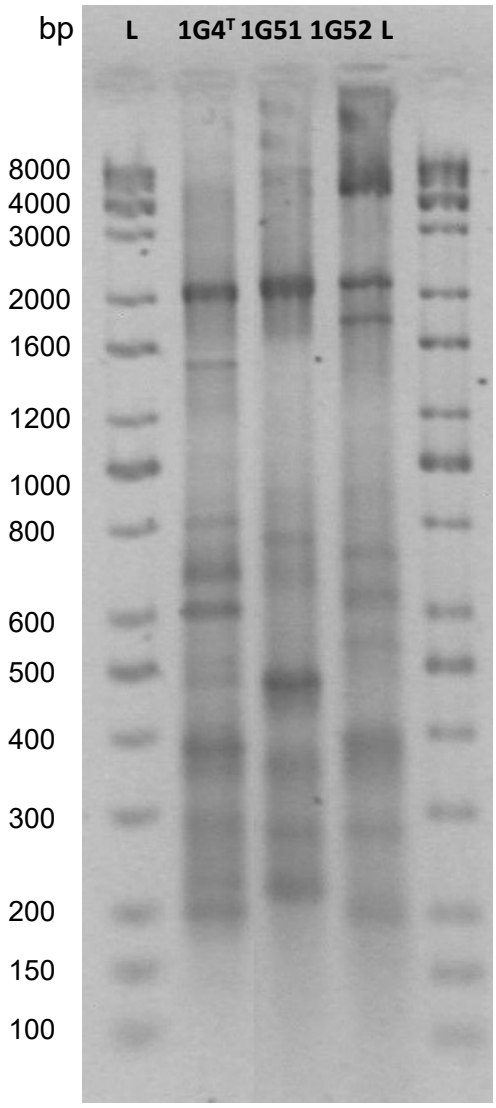
Key: +, positive; −, negative.

*polar lipids data for strain 2 are from Busarakam *et al*. [10]; for 3 from Golinska *et al*. [3], 4,7, 8 and 10 from Montero-Calasanz *et al*. [4]; for 5 from Zhang *et al*. [23]; for 6 and 9 from Trujillo *et al*. [8].

Codes: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; GPI, glycophosphoinositol; PIM, phosphoinositolmannoside; AL, aminolipids; APL, aminophospholipids; GL, glycolipid; PL, unidentified phospholipid; L, unidentified lipid.

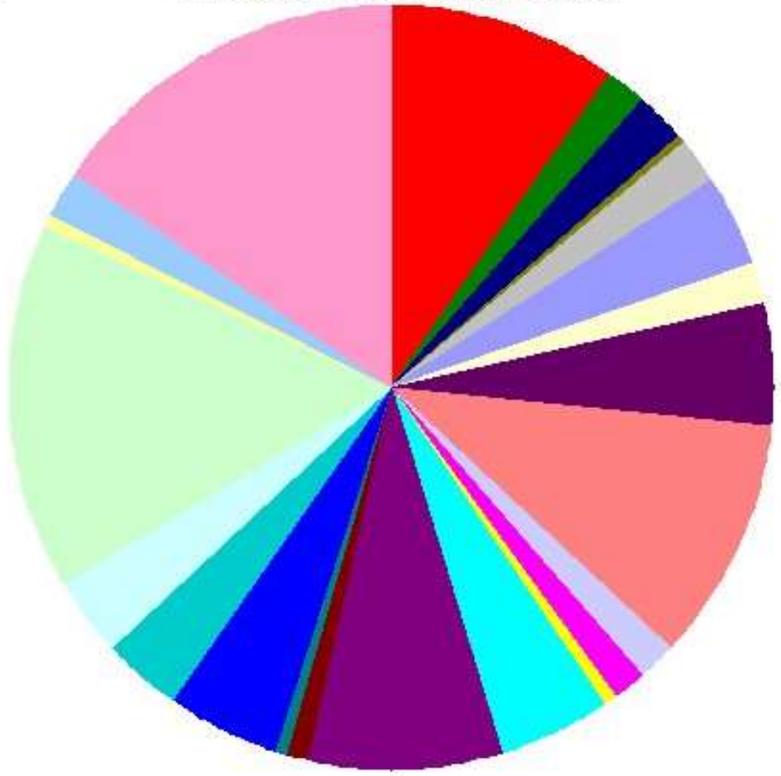
All of the strains oxidised D-glucose, but not D-arabitol, D-aspartic acid, L-histidine, mucic acid or D-serine. Between one and three of the strains gave variable results in the duplicated tests, involving oxidation of L-alanine and L-galactonic acid-γ-lactone and growth in the presence of lithium chloride.


0.007
-/-
Isolate 1G51 (MH430522)
-/96
Isolate 1G52 (MN065570)
-/96
Isolate 1G4T (MH430521)
71/96
'Modestobacter excelsi' DSM 107535PT (MH430528)
Modestobacter italicus BC 501T (MK020151)
-/96
Modestobacter roseus DSM 45764 T (JQ819258)
Modestobacter marinus DSM 45201T (EU181225)
88/100
Modestobacter caceresii KNN 45-2bT (LN898173)
-/100
Modestobacter lacusdianchii KCTC 39600T (KP986567)
-/100
-/98
Modestobacter versicolor DSM 16678T (AJ871304)
Modestobacter muralis MDVD1T (LN810545)
-/100
Modestobacter multiseptatus DSM 44406T (Y18646)
Modestobacter lapidis MON 3.1T (LN810544)
71/100
Klenkia marina DSM 45722T (LT746188)
99/100
Geodermatophilus obscurus DSM 43160T (CP001867)
Blastococcus aggregatus ATCC 25902T (NR 025925)
Cumulibacter manganitolerans 2-36T (KX602199)


bp
L
1G4T
1G51
1G52
L
8000
4000
3000
2000
1600
1200
1000
800
600
500
400
300
200
150
100

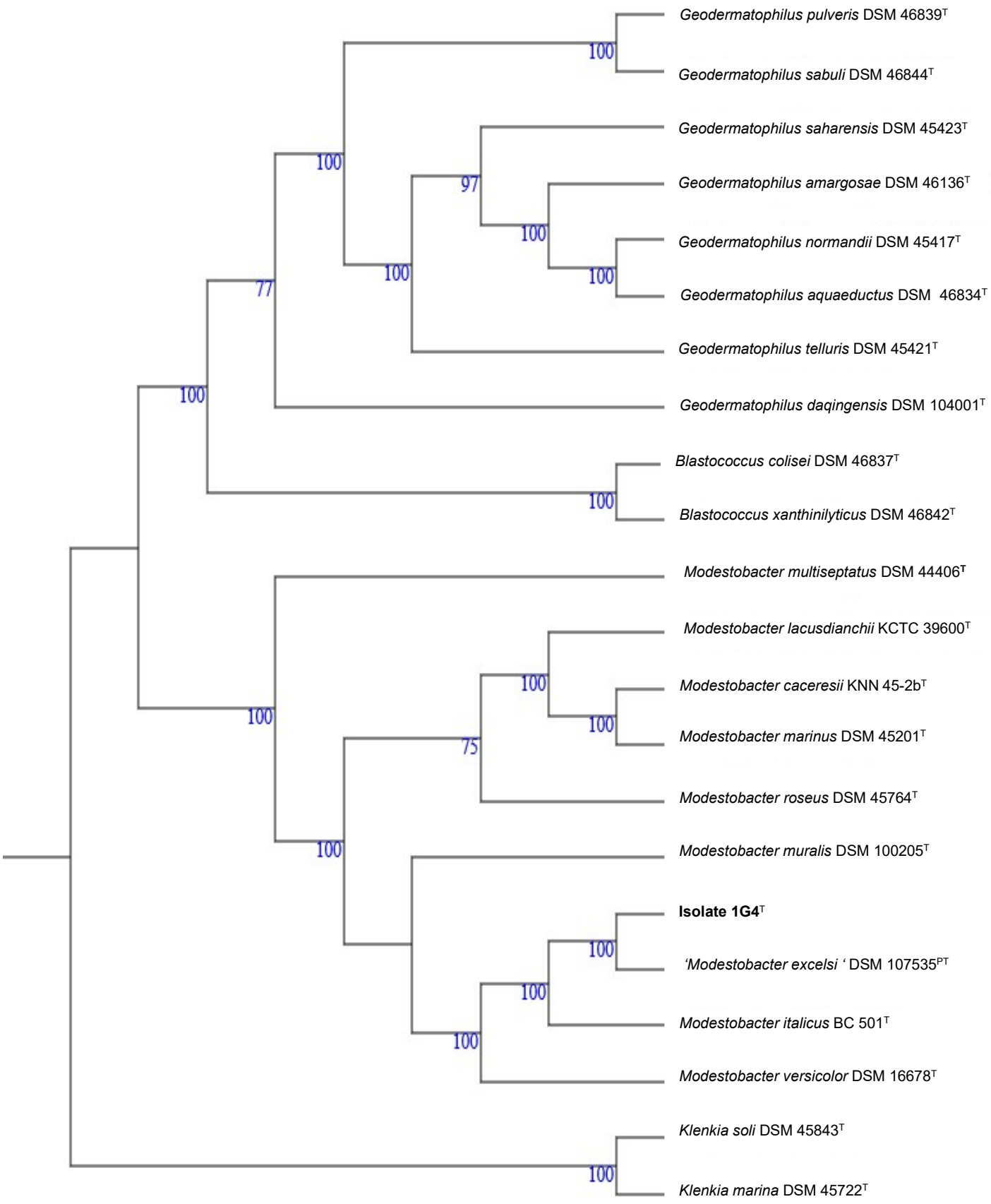
Subsystem Coverage

Subsystem Category Distribution



Subsystem Feature Counts

- Cofactors, Vitamins, Prosthetic Groups, Pigments (197)
- Cell Wall and Capsule (33)
- Virulence, Disease and Defense (43)
- Potassium metabolism (7)
- Photosynthesis (0)
- Miscellaneous (40)
- Phages, Prophages, Transposable elements, Plasmids (0)
- Membrane Transport (74)
- Iron acquisition and metabolism (4)
- RNA Metabolism (36)
- Nucleosides and Nucleotides (105)
- Protein Metabolism (201)
- Cell Division and Cell Cycle (0)
- Motility and Chemotaxis (36)
- Regulation and Cell signaling (23)
- Secondary Metabolism (12)
- DNA Metabolism (94)
- Fatty Acids, Lipids, and Isoprenoids (166)
- Nitrogen Metabolism (18)
- Dormancy and Sporulation (9)
- Respiration (97)
- Stress Response (64)
- Metabolism of Aromatic Compounds (65)
- Amino Acids and Derivatives (311)
- Sulfur Metabolism (13)
- Phosphorus Metabolism (36)
- Carbohydrates (303)


Geodermatophilus pulveris DSM 46839T
Geodermatophilus sabuli DSM 46844T
Geodermatophilus saharensis DSM 45423T
Geodermatophilus amargosae DSM 46136T
Geodermatophilus normandii DSM 45417T
Geodermatophilus aquaeductus DSM 46834T
Geodermatophilus telluris DSM 45421T
Geodermatophilus daqingensis DSM 104001T
Blastococcus colisei DSM 46837T
Blastococcus xanthinilyticus DSM 46842T
Modestobacter multiseptatus DSM 44406T
Modestobacter lacusdianchii KCTC 39600T
Modestobacter caceresii KNN 45-2bT
Modestobacter marinus DSM 45201T
Modestobacter roseus DSM 45764T
Modestobacter muralis DSM 100205T
Isolate 1G4T
'Modestobacter excelsi ' DSM 107535PT
Modestobacter italicus BC 501T
Modestobacter versicolor DSM 16678T
Klenkia soli DSM 45843T
Klenkia marina DSM 45722T
100
100
97
100
100
100
77
100
100
100
100
100
75
100
100
100
100
100

Modestobacter altitudinis sp. nov., a novel actinobacterium isolated from Atacama Desert soil

Patrycja Golinska, Magdalena Świecimska, Maria del Carmen Montero-Calasanz, Adnan Yaramis, Jose M. Igual, Alan T. Bull, Michael Goodfellow

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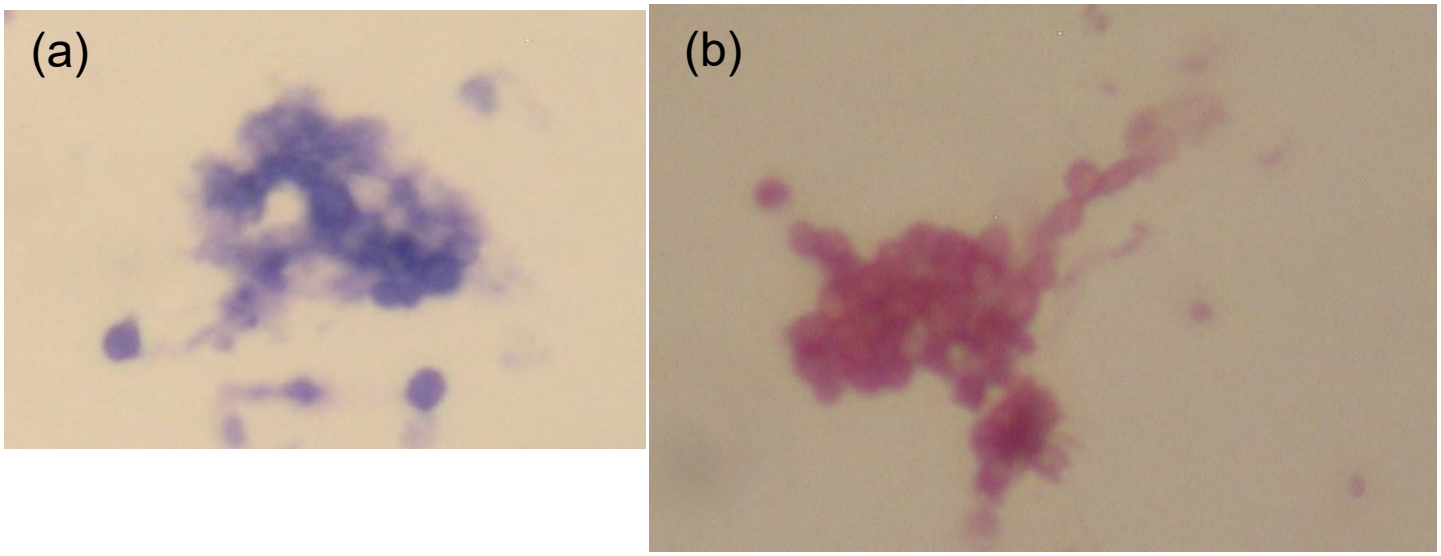

Fig. S1. Micromorphology of isolate 1G4^{T} stained with (a) methylene blue and (b) fuchsin.

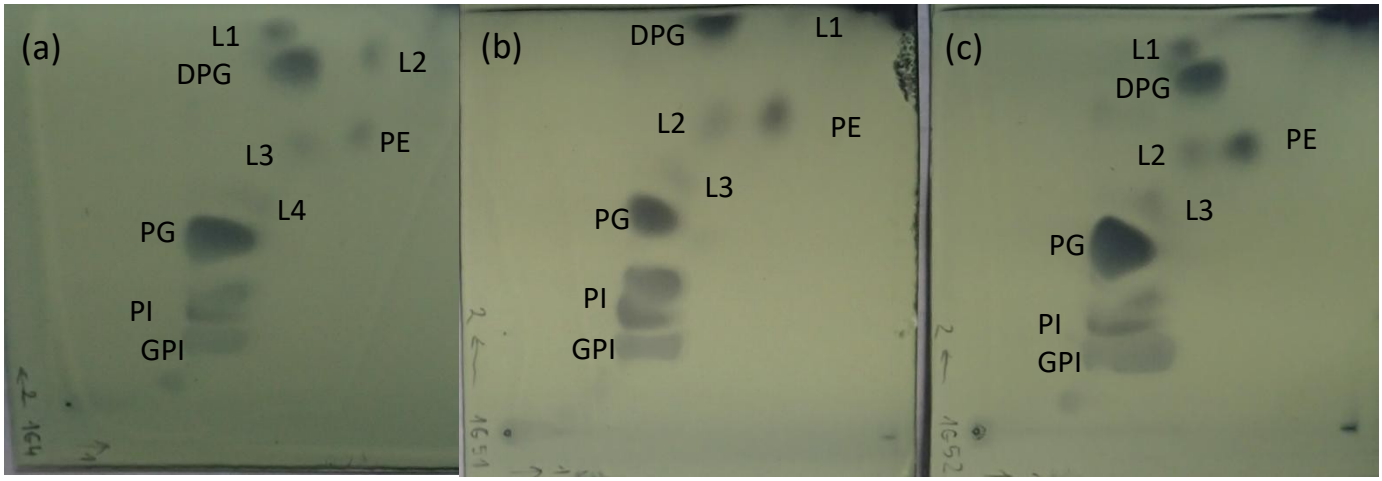

Fig. S2. Polar lipids profile of strains (a) 1G4^{T}, (b) 1G51 and (c) 1G52 after separation by two-dimensional TLC using chloroform:methanol:water (65 : 25 : 4; by volume) in the first dimension and chloroform:methanol:acetic acid:water (80 : 12 : 15 : 4; by volume) in the second dimension. Plates were sprayed with molybdenum blue (Sigma) for the detection of total polar lipids. DPG, diphosphadidylglycerol; GPI, glycophosphatidylinositol; PE, phosphatidethanolamine; PG, phosphadidylglycerol; PI, phosphatidylinositol; L1–4, unidentified lipids.

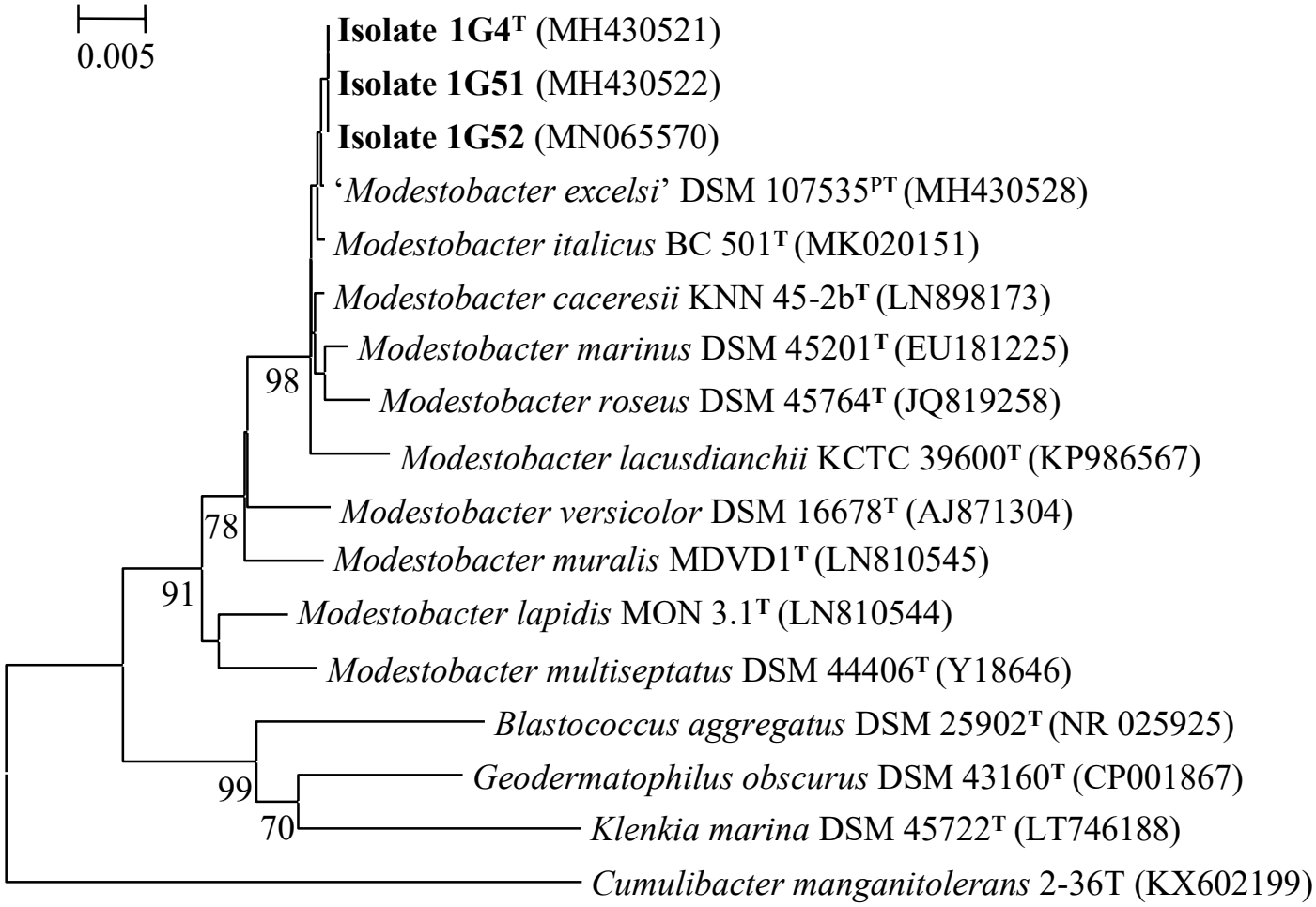

Fig. S3. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1390-1541) showing relationships between isolates 1G4^{T}, 1G51 and 1G52 and between them and the type strains of *Modestobacter* species. The numbers at the nodes are bootstrap support values over 70%. Bar; 0.005 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^{T} as the outgroup.